

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
 United States Patent and Trademark
 Office
 Box PCT
 Washington, D.C.20231
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 October 2000 (23.10.00)	
International application No. PCT/EP00/01587	Applicant's or agent's file reference FB/BC45224
International filing date (day/month/year) 28 February 2000 (28.02.00)	Priority date (day/month/year) 05 March 1999 (05.03.99)
Applicant VINALS y de BASSOLS, Carlota	

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

21 September 2000 (21.09.00)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Pascal Piriou Telephone No.: (41-22) 338.83.38
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PACIENT COOPERATION TREAT

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION RELATING TO PRIORITY CLAIM

(PCT Rules 26bis.1 and 26bis.2 and
Administrative Instructions, Sections 402 and 409)

To:

PRIVETT, Kathryn, Louise
SmithKline Beecham
Corporate Intellectual Property
Two New Horizons Court
Brentford
Middlesex TW8 9EP
ROYAUME-UNI

Date of mailing (day/month/year) 17 July 2000 (17.07.00)	
Applicant's or agent's file reference FB/BC45224	IMPORTANT NOTIFICATION
International application No. PCT/EP00/01587	International filing date (day/month/year) 28 February 2000 (28.02.00)
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A. et al	

The applicant is hereby notified of the following in respect of the priority claim(s) made in the international application.

1. ☒ **Correction of priority claim.** In accordance with the applicant's notice received on: 23 June 2000 (23.06.00), the following priority claim has been corrected to read as follows:
GB 05 March 1999 (05.03.99) 9905124.5
☐ even though the indication of the number of the earlier application is missing.
☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:
2. ☐ **Addition of priority claim.** In accordance with the applicant's notice received on: , the following priority claim has been added:
☐ even though the indication of the number of the earlier application is missing.
☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:
3. ☒ As a result of the correction and/or addition of (a) priority claim(s) under items 1 and/or 2, the (earliest) priority date is:
05 March 1999 (05.03.99)
4. ☐ **Priority claim considered not to have been made.**
☐ The applicant failed to respond to the Invitation under Rule 26bis.2(a) (Form PCT/IB/316) within the prescribed time limit.
☐ The applicant's notice was received after the expiration of the prescribed time limit under Rule 26bis.1(a).
☐ The applicant's notice failed to correct the priority claim so as to comply with the requirements of Rule 4.10.
The applicant may, before the technical preparations for international publication have been completed and subject to the payment of a fee, request the International Bureau to publish, together with the international application, information concerning the priority claim. See Rule 26bis.2(c) and the PCT Applicant's Guide, Volume I, Annex B2(IB).
5. ☐ In case where multiple priorities have been claimed, the above item(s) relate to the following priority claim(s):
6. A copy of this notification has been sent to the receiving Office and
☒ to the International Searching Authority (where the international search report has not yet been issued).
☒ the designated Offices (which have already been notified of the receipt of the record copy).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Aino Metcalfe
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

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PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

GIDDINGS, Peter John
GLAXOSMITHKLINE
Corporate Intellectual Property
Two New Horizons Court
Brentford
Middlesex TW8 9EP
GRANDE BRETAGNE

RECEIVED

11 JUN 2001

NEW HORIZONS COURT

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing
(day/month/year)

07.06.2001

Applicant's or agent's file reference
FB/BC45224

IMPORTANT NOTIFICATION

International application No.
PCT/EP00/01587

International filing date (day/month/year)
28/02/2000

Priority date (day/month/year)
05/03/1999

Applicant

SMITHKLINE BEECHAM BIOLOGICALS S.A.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

SIEDSMA, Y

Tel. +49 89 2399-8162





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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference FB/BC45224		FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP00/01587	International filing date (day/month/year) 28/02/2000	Priority date (day/month/year) 05/03/1999	
International Patent Classification (IPC) or national classification and IPC A61K38/17			
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A.			
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 9 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 1 sheets.</p>			
<p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the report</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input checked="" type="checkbox"/> Certain observations on the international application</p>			
Date of submission of the demand 21/09/2000		Date of completion of this report 07.06.2001	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized officer Favre, N Telephone No. +49 89 2399 7363 	

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01587

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-43 as originally filed

Claims, No.:

1-12 as originally filed

13-16 with telefax of 10/04/2001

Sequence listing part of the description, pages:

1-9, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/01587

- ☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
☒ claims Nos. 9-11 and 12 a) and c).

because:

- ☒ the said international application, or the said claims Nos. 9 and 10 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 11 and 12 a) and c).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

- ☐ the written form has not been furnished or does not comply with the standard.
☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/01587

Novelty (N)	Yes:	Claims	1-10, 12b) and 13-16
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10, 12b) and 13-16
Industrial applicability (IA)	Yes:	Claims	1-8, 12b) and 13-16
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

se separate sheet

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Re It m III**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. Claims 9 and 10 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).
2. Claims 11, 12a) and 12c) relate to subject-matter which was considered to lack support and sufficiency of disclosure in the sense of Articles 5 and 6 PCT by the International Search Authority and which has therefore not been searched. Pursuant to Rule 66.1(e) PCT, no opinion will be formulated with respect to novelty, inventive step and industrial applicability of the subject-matter of these claims.

Re Item V**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. For the assessment of the present claims 9 and 10 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

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2. Document D1 (Cancer Research, 1994, **54**:3645-3650) discloses (cf. abstract and Fig. 1) that the ERK gene encoding for a receptor protein-tyrosine kinase, which gene is also known as EPHB2, EPHB2v, EBHB2, EPH3, EPHT3, DRT, HEK5 or CASB616, and which product is a polypeptide having an amino acid sequence corresponding to SEQ ID NO: 2 or 4, that is encoded by a nucleotide sequence corresponding to SEQ ID NO: 1 or 3, is overexpressed in various tumours of epithelial origins (among others colon cancer, e.g. Fig 2). Moreover, D1 discloses that ERK might play a significant role in carcinogenesis.

Document D2 (Biochemical and Biophysical Research Communications, 1993, **194**(2):698-705) discloses (cf. abstract and Fig. 1) that the ERK gene is overexpressed in human gastric cancer tissues and might play a significant role in carcinogenesis of the stomach.

Document D3 (Human Molecular Genetics, 1995, **4**(11):2033-2045) discloses (cf. abstract and Fig. 1) that the DRT gene is overexpressed in a large number of tumour cell lines derived from neuroectoderm and in small cell lung carcinoma (SCLC). Moreover, D3 discloses that this gene might play a significant role in neuroblastoma and SCLC tumorigenesis.

Document D4 (Oncogene, 1995, **10**:897-905) analyses the expression of the HEK5 gene and discloses that this gene is abundantly expressed in thyroid, colon and tumour cells (Fig. 1 and page 902, column 1, lines 5-18).

- 2.1 Independent claim 1 defines a vaccine composition comprising a polypeptide which has at least 85% identity with the product of the ERK (=DRT, =HEK5) gene. In other words, the subject-matter of independent claim 1 differs from the disclosures of D1-D4 in that the overexpressed ERK is used in a vaccine composition.
- 2.2 The problem to be solved by the present invention may therefore be regarded as the provision of a vaccine for the treatment of diseases, e.g. cancers.
- 2.3 For the person skilled in the art, it is obvious that the product of a gene which is overexpressed in a pathological condition is an excellent candidate for designing a

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vaccine against said pathological condition. Hence the skilled person would consider the polypeptides of D1-D4 for use in a vaccine composition.

Moreover, there is no evidence in the present application that would convincingly demonstrate that **any** of the vaccine compositions encompassed within the broad wording of independent claim 1 effectively induces of a protective immune response against a pathogenic condition. Therefore, the subject-matter of independent claim 1 cannot be considered as being a solution to the above-stated technical problem, and as such cannot be considered as being inventive.

Therefore, the subject-matter of independent claim 1 is not inventive in the sense of Article 33(3) PCT.

- 2.4 A similar argumentation applies for the subject-matter of independent claim 3 which defines a vaccine composition comprising a polynucleotide which has at least 85% identity with the ERK gene. Claim 3 thus lacks inventive step in the sense of Article 33(3) PCT.
- 2.5 The same applies for independent claim 4 which defines a vaccine comprising antigen presenting cells loaded with the product of the ERK gene or genetically modified to express such product. Claim 4 thus also lacks inventive step in the sense of Article 33(3) PCT.
- 2.6 Dependent claims 2, 5 and 6 further define standard embodiments of the independent claims to which they refer and do not appear to contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step (Article 33(3) PCT).
3. Generating antibodies against a known antigen does not require an inventive activity from the skilled person. Moreover, according to the arguments of 2.-2.7 the skilled person would be prompted by the disclosure of D1-D4 to generate said antibodies, either for therapeutic or for diagnostic purposes. Finally, it does not appear that there is an unexpected effect associated with any of the antibodies encompassed within the wording of claim 7. Independent claim 7 is therefore not inventive in the sense of Article 33(3) PCT.

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- 3.1 In addition, in the view of the disclosed strong relationship between ERK expression and cancerogenesis, the skilled person would also attempt to identify compounds that modulate the function of said gene or of the product of said gene. Hence, claim 8 is not inventive in the sense of Article 33(3) PCT.
- 3.2 The same applies for the methods of treatment defined in claims 9 and 10. Said claims thus also lack inventive step in the sense of Article 33(3) PCT.
4. Documents D1-D4 disclose isolated polynucleotides highly similar to those defined by SEQ ID NO: 1 or 3. Given that no unexpected effect seems to be associated with the particular polynucleotides of SEQ ID NO: 1 or 3, the subject-matter of claim 12b) is not considered to be inventive in the sense of Article 33(3) PCT.
5. Finally, in the view of the arguments of 2.-2.7, it is obvious for the person skilled in the art that either the ERK gene or the product of said gene can be used for diagnosing a disease or a susceptibility to a disease, more particularly of colon or gastric cancer. The subject-matter of independent claims 13-16 is hence not inventive in the sense of Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. The term "vaccine" implies the generation of a **protective immune response** against a pathogen or against a pathogenic condition. However, only a minority of antigen are actually able to induce such a **protective immune response**, although all antigens are immunogenic per definition. In the application as filed, there is no experimental data convincingly supporting the claimed vaccines and methods of treatment.

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Moreover, when a given technical effect, e.g. immunoprotection *in vivo*, is solely relied upon in order to demonstrate that a claimed subject-matter, e.g. a vaccine against a pathogenic condition, involves an inventive step, claims of a broad scope are not allowable when, on the basis of the original disclosure and of the common general knowledge at the date of filing or at the priority date, said technical effect cannot be achieved by the skilled person without undue burden, i.e. the determination of the effective epitopes, within the whole range of application claimed.

Therefore, claims 1-6, 9 and 10 are not considered to be supported by the description in the sense of Article 6 PCT.

- 1.1 Moreover, in the light of the above objection, it appears that the subject-matter of claims 1-6, 9 and 10 is purely **theoretical** and **speculative**. The attention of the applicant is drawn to the fact that most national and regional Offices will not deliver patents for speculative subject-matter.
- 1.2 In addition, the attention of the applicant is drawn to the fact that it is well accepted that the protection conferred by a patent should correspond to the **technical** contribution to the art made by the disclosure of the invention described therein. From the description (page 2, lines 20-24 and Example 1), it appears that said contribution is the recognition that ERK is overexpressed in tumours, as compared to normal tissue. This however has already been disclosed in D1-D4.

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REC'D 11 JUN 2001

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference FB/BC45224		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) FOR FURTHER ACTION	
International application No. PCT/EP00/01587	International filing date (day/month/year) 28/02/2000	Priority date (day/month/year) 05/03/1999	
International Patent Classification (IPC) or national classification and IPC A61K38/17			
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 9 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 21/09/2000	Date of completion of this report 07.06.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Favre, N Telephone No. +49 89 2399 7363 

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01587

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-43 as originally filed

Claims, No.:

1-12 as originally filed

13-16 with telefax of 10/04/2001

Sequence listing part of the description, pages:

1-9, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01587

- ☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
☒ claims Nos. 9-11 and 12 a) and c).

because:

- ☒ the said international application, or the said claims Nos. 9 and 10 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 11 and 12 a) and c).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

- ☐ the written form has not been furnished or does not comply with the standard.
☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01587

Novelty (N)	Yes:	Claims	1-10, 12b) and 13-16
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10, 12b) and 13-16
Industrial applicability (IA)	Yes:	Claims	1-8, 12b) and 13-16
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

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Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 9 and 10 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).
2. Claims 11, 12a) and 12c) relate to subject-matter which was considered to lack support and sufficiency of disclosure in the sense of Articles 5 and 6 PCT by the International Search Authority and which has therefore not been searched. Pursuant to Rule 66.1(e) PCT, no opinion will be formulated with respect to novelty, inventive step and industrial applicability of the subject-matter of these claims.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. For the assessment of the present claims 9 and 10 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

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2. Document D1 (Cancer Research, 1994, 54:3645-3650) discloses (cf. abstract and Fig. 1) that the ERK gene encoding for a receptor protein-tyrosine kinase, which gene is also known as EPHB2, EPHB2v, EBHB2, EPH3, EPHT3, DRT, HEK5 or CASB616, and which product is a polypeptide having an amino acid sequence corresponding to SEQ ID NO: 2 or 4, that is encoded by a nucleotide sequence corresponding to SEQ ID NO: 1 or 3, is overexpressed in various tumours of epithelial origins (among others colon cancer, e.g. Fig 2). Moreover, D1 discloses that ERK might play a significant role in carcinogenesis.

Document D2 (Biochemical and Biophysical Research Communications, 1993, 194(2):698-705) discloses (cf. abstract and Fig. 1) that the ERK gene is overexpressed in human gastric cancer tissues and might play a significant role in carcinogenesis of the stomach.

Document D3 (Human Molecular Genetics, 1995, 4(11):2033-2045) discloses (cf. abstract and Fig. 1) that the DRT gene is overexpressed in a large number of tumour cell lines derived from neuroectoderm and in small cell lung carcinoma (SCLC). Moreover, D3 discloses that this gene might play a significant role in neuroblastoma and SCLC tumorigenesis.

Document D4 (Oncogene, 1995, 10:897-905) analyses the expression of the HEK5 gene and discloses that this gene is abundantly expressed in thyroid, colon and tumour cells (Fig. 1 and page 902, column 1, lines 5-18).

- 2.1 Independent claim 1 defines a vaccine composition comprising a polypeptide which has at least 85% identity with the product of the ERK (=DRT, =HEK5) gene. In other words, the subject-matter of independent claim 1 differs from the disclosures of D1-D4 in that the overexpressed ERK is used in a vaccine composition.
- 2.2 The problem to be solved by the present invention may therefore be regarded as the provision of a vaccine for the treatment of diseases, e.g. cancers.
- 2.3 For the person skilled in the art, it is obvious that the product of a gene which is overexpressed in a pathological condition is an excellent candidate for designing a

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vaccine against said pathological condition. Hence the skilled person would consider the polypeptides of D1-D4 for use in a vaccine composition. Moreover, there is no evidence in the present application that would convincingly demonstrate that **any** of the vaccine compositions encompassed within the broad wording of independent claim 1 effectively induces of a protective immune response against a pathogenic condition. Therefore, the subject-matter of independent claim 1 cannot be considered as being a solution to the above-stated technical problem, and as such cannot be considered as being inventive. Therefore, the subject-matter of independent claim 1 is not inventive in the sense of Article 33(3) PCT.

- 2.4 A similar argumentation applies for the subject-matter of independent claim 3 which defines a vaccine composition comprising a polynucleotide which has at least 85% identity with the ERK gene. Claim 3 thus lacks inventive step in the sense of Article 33(3) PCT.
- 2.5 The same applies for independent claim 4 which defines a vaccine comprising antigen presenting cells loaded with the product of the ERK gene or genetically modified to express such product. Claim 4 thus also lacks inventive step in the sense of Article 33(3) PCT.
- 2.6 Dependent claims 2, 5 and 6 further define standard embodiments of the independent claims to which they refer and do not appear to contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step (Article 33(3) PCT).
3. Generating antibodies against a known antigen does not require an inventive activity from the skilled person. Moreover, according to the arguments of 2.-2.7 the skilled person would be prompted by the disclosure of D1-D4 to generate said antibodies, either for therapeutic or for diagnostic purposes. Finally, it does not appear that there is an unexpected effect associated with any of the antibodies encompassed within the wording of claim 7. Independent claim 7 is therefore not inventive in the sense of Article 33(3) PCT.

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- 3.1 In addition, in the view of the disclosed strong relationship between ERK expression and cancerogenesis, the skilled person would also attempt to identify compounds that modulate the function of said gene or of the product of said gene. Hence, claim 8 is not inventive in the sense of Article 33(3) PCT.
- 3.2 The same applies for the methods of treatment defined in claims 9 and 10. Said claims thus also lack inventive step in the sense of Article 33(3) PCT.
4. Documents D1-D4 disclose isolated polynucleotides highly similar to those defined by SEQ ID NO: 1 or 3. Given that no unexpected effect seems to be associated with the particular polynucleotides of SEQ ID NO: 1 or 3, the subject-matter of claim 12b) is not considered to be inventive in the sense of Article 33(3) PCT.
5. Finally, in the view of the arguments of 2.-2.7, it is obvious for the person skilled in the art that either the ERK gene or the product of said gene can be used for diagnosing a disease or a susceptibility to a disease, more particularly of colon or gastric cancer. The subject-matter of independent claims 13-16 is hence not inventive in the sense of Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. The term "vaccine" implies the generation of a **protective immune response** against a pathogen or against a pathogenic condition. However, only a minority of antigen are actually able to induce such a **protective immune response**, although all antigens are immunogenic per definition. In the application as filed, there is no experimental data convincingly supporting the claimed vaccines and methods of treatment.

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Moreover, when a given technical effect, e.g. immunoprotection *in vivo*, is solely relied upon in order to demonstrate that a claimed subject-matter, e.g. a vaccine against a pathogenic condition, involves an inventive step, claims of a broad scope are not allowable when, on the basis of the original disclosure and of the common general knowledge at the date of filing or at the priority date, said technical effect cannot be achieved by the skilled person without undue burden, i.e. the determination of the effective epitopes, within the whole range of application claimed.

Therefore, claims 1-6, 9 and 10 are not considered to be supported by the description in the sense of Article 6 PCT.

- 1.1 Moreover, in the light of the above objection, it appears that the subject-matter of claims 1-6, 9 and 10 is purely **theoretical** and **speculative**. The attention of the applicant is drawn to the fact that most national and regional Offices will not deliver patents for speculative subject-matter.
- 1.2 In addition, the attention of the applicant is drawn to the fact that it is well accepted that the protection conferred by a patent should correspond to the **technical** contribution to the art made by the disclosure of the invention described therein. From the description (page 2, lines 20-24 and Example 1), it appears that said contribution is the recognition that ERK is overexpressed in tumours, as compared to normal tissue. This however has already been disclosed in D1-D4.

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PATENT COOPERATION TREA

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

To:
SmithKline Beecham
Corporate Intellectual Property
Attn. PRIVETT, Kathryn L.
Two New Horizons Court
Brentford
Middlesex TW8 9EP
UNITED KINGDOM

Date of mailing (day/month/year)	26/10/2000
-------------------------------------	------------

Applicant's or agent's file reference FB/BC45224	FOR FURTHER ACTION See paragraphs 1 and 4 below
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International application No. PCT/EP 00/01587	International filing date (day/month/year) 28/02/2000
---	---

Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A.

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.
Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.


☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority  European Patent Office, P.B. 5818 Patentaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Catherine Humbert
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NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

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NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

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PATENT COOPERATION TREA .

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference FB/BC45224	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 00/ 01587	International filing date (day/month/year) 28/02/2000	(Earliest) Priority Date (day/month/year) 05/03/1999
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (see Box II).

4. With regard to the title,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

USE OF CASB616 POLYPEPTIDES AND POLYNUCLEOTIDES FOR CANCER TREATMENT

5. With regard to the abstract,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



None of the figures.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01587

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	A61K38/17	A61K39/395	C07K14/705	A61P35/00	A61K39/00
	A61K48/00	C07K16/28	G01N33/68	C12Q1/68	C12N15/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KIYOKAWA E ET AL: "OVEREXPRESSION OF ERK AN EPH FAMILY RECEPTOR PROTEIN TYROSINE KINASE IN VARIOUS HUMAN TUMORS" CANCER RESEARCH, US, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, vol. 54, 15 July 1994 (1994-07-15), pages 3645-3650, XP002070092 ISSN: 0008-5472	14, 16
Y	the whole document — -/-	1-7

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

10 October 2000

Date of mailing of the international search report

26/10/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Noë, V

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01587

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>CHEN YING ET AL: "DNA vaccines encoding full-length or truncated Neu induce protective immunity against Neu-expressing mammary tumors." CANCER RESEARCH, vol. 58, no. 9, 1 May 1998 (1998-05-01), pages 1965-1971, XP002149613 ISSN: 0008-5472 abstract page 1965, column 1, last paragraph -column 2, paragraph 3 page 1967, column 2, last paragraph -page 1969, column 1, paragraph 2; figures 4-6</p>	1-7
A	<p>IWASE T ET AL: "IDENTIFICATION OF PROTEIN-TYROSINE KINASE GENES PREFERENTIALLY EXPRESSED IN EMBRYO STOMACH AND GASTRIC CANCER" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,US,ACADEMIC PRESS INC. ORLANDO, FL, vol. 194, no. 2, 30 July 1993 (1993-07-30), pages 698-705, XP002014925 ISSN: 0006-291X abstract figures 1,3B page 704, paragraph 2 - paragraph 3 page 704, last paragraph</p>	1-4,7,9, 10,12, 14,16
A	<p>IKEGAKI NAOHIKO ET AL: "Molecular characterization and chromosomal localization of DRT (EPHT3): A developmentally regulated human protein-tyrosine kinase gene of the EPH family." HUMAN MOLECULAR GENETICS, vol. 4, no. 11, 1995, pages 2033-2045, XP000946771 ISSN: 0964-6906 abstract figure 1 page 2037, column 1, paragraph 2 -page 2038, column 1, paragraph 1 page 2042, column 1, paragraph 3 -column 2, paragraph 1</p> <p style="text-align: center;">-/-</p>	1-4,7,9, 12-16

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01587

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>FOX GARY M ET AL: "CDNA cloning and tissue distribution of five human EPH-like receptor protein-tyrosine kinases." ONCOGENE, vol. 10, no. 5, 1995, pages 897-905, XP000946743 ISSN: 0950-9232 abstract page 898, column 1, line 2 - line 6 page 902, column 1, line 15 - line 17 figure 1</p> <p>-----</p>	1-4,7,9, 10,12-16

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 11,12 (a and c)

Present claims 11,12(a and c) relate to compounds defined by reference to a desirable characteristic or property, namely Erk agonist or antagonist and nucleic acids modulating Erk expression.

The claims cover all products having these characteristics, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the products by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to Erk polypeptides and polynucleotides.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/01587

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 9-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 11,12 (a and c)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01587

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/17 A61K39/395 C07K14/705 A61P35/00 A61K39/00
A61K48/00 C07K16/28 G01N33/68 C12Q1/68 C12N15/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KIYOKAWA E ET AL: "OVEREXPRESSION OF ERK AN EPH FAMILY RECEPTOR PROTEIN TYROSINE KINASE IN VARIOUS HUMAN TUMORS" CANCER RESEARCH, US, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, vol. 54, 15 July 1994 (1994-07-15), pages 3645-3650, XP002070092 ISSN: 0008-5472	14, 16
Y	the whole document --- -/--	1-7

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Δ" document member of the same patent family

Date of the actual completion of the international search

10 October 2000

Date of mailing of the international search report

26/10/2000

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Noë, V.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/01587

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEN YING ET AL: "DNA vaccines encoding full-length or truncated Neu induce protective immunity against Neu-expressing mammary tumors." CANCER RESEARCH, vol. 58, no. 9, 1 May 1998 (1998-05-01), pages 1965-1971, XP002149613 ISSN: 0008-5472 abstract page 1965, column 1, last paragraph -column 2, paragraph 3 page 1967, column 2, last paragraph -page 1969, column 1, paragraph 2; figures 4-6 ---	1-7
A	IWASE T ET AL: "IDENTIFICATION OF PROTEIN-TYROSINE KINASE GENES PREFERENTIALLY EXPRESSED IN EMBRYO STOMACH AND GASTRIC CANCER" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,US,ACADEMIC PRESS INC. ORLANDO, FL, vol. 194, no. 2, 30 July 1993 (1993-07-30), pages 698-705, XP002014925 ISSN: 0006-291X abstract figures 1,3B page 704, paragraph 2 - paragraph 3 page 704, last paragraph ---	1-4,7,9, 10,12, 14,16
A	IKEGAKI NAOHIKO ET AL: "Molecular characterization and chromosomal localization of DRT (EPHT3): A developmentally regulated human protein-tyrosine kinase gene of the EPH family." HUMAN MOLECULAR GENETICS, vol. 4, no. 11, 1995, pages 2033-2045, XP000946771 ISSN: 0964-6906 abstract figure 1 page 2037, column 1, paragraph 2 -page 2038, column 1, paragraph 1 page 2042, column 1, paragraph 3 -column 2, paragraph 1 --- -/--	1-4,7,9, 12-16

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 00/01587

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>FOX GARY M ET AL: "CDNA cloning and tissue distribution of five human EPH-like receptor protein-tyrosine kinases." ONCOGENE, vol. 10, no. 5, 1995, pages 897-905, XP000946743 ISSN: 0950-9232 abstract page 898, column 1, line 2 - line 6 page 902, column 1, line 15 - line 17 figure 1</p> <p style="text-align: center;">-----</p>	<p>1-4, 7, 9, 10, 12-16</p>

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 11,12 (a and c)

Present claims 11,12(a and c) relate to compounds defined by reference to a desirable characteristic or property, namely Erk agonist or antagonist and nucleic acids modulating Erk expression.

The claims cover all products having these characteristics, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the products by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to Erk polypeptides and polynucleotides.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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NEW HORIZONS COURT

(54) Title: **USE OF CASB616 POLYPEPTIDES AND POLYNUCLEOTIDES FOR CANCER TREATMENT**

(57) Abstract: Use of CASB616 polypeptides and polynucleotides in diagnostics, and vaccines for prophylactic and therapeutic treatment of cancers, particularly colon cancers, autoimmune diseases, and related conditions.

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(54) Title: NOVEL USES (57) Abstract Use of CASB616 polypeptides and polynucleotides in diagnostics, and vaccines for prophylactic and therapeutic treatment of cancers, particularly colon cancers, autoimmune diseases, and related conditions.		

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Novel Uses

The present invention relates to methods for using polypeptides and polynucleotides (herein referred to as "CASB616" polypeptide(s) and "CASB616" polynucleotide(s)"), including the treatment of cancer and autoimmune diseases and other related conditions. In a further aspect, the invention relates to methods for identifying agonists and antagonists/inhibitors using the materials provided by the invention, and treating conditions associated with CASB616 polypeptide imbalance with the identified compounds. In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with inappropriate CASB616 polypeptide activity or levels.

It has been discovered that CASB616 encodes a polypeptide which belongs to the largest family of receptor protein-tyrosine kinases, the EPH and EPH-related receptors. CASB616 is otherwise known as EPHB2 (aliases: EBHB2, , ERK, EPH3, EPHT3, DRT, HEK5) and EPHB2v.

The EPH and EPH-related receptors have been implicated in mediating developmental events, particularly in the nervous system. Receptors in the Eph subfamily typically have a single kinase domain and an extracellular region containing a Cys-rich domain and 2 fibronectin type III repeats. The ligands for Eph receptors have been named ephrins by the Eph Nomenclature Committee (Cell 90: 403-404, 1997; PubMed ID : 9267020). Based on their structures and sequence relationships, ephrins are divided into the ephrin-A (EFNA) class, which are anchored to the membrane by a glycosylphosphatidylinositol linkage, and the ephrin-B (EFNB) class, which are transmembrane proteins. The Eph family of receptors are divided into 2 groups based on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands. The Eph Nomenclature Committee (1997) proposed that Eph receptors interacting preferentially with ephrin-A proteins be called EphA and Eph receptors interacting preferentially with ephrin-B proteins be called EphB.

Ikegaki *et al.* (Hum. Molec. Genet. 4: 2033-2045, 1995) mapped DRT, the EPHB2 gene, to 1p36.1-p35 by PCR screening of human/rodent somatic cell hybrid panels and by fluorescence in situ hybridization. As the distal end of 1p is often deleted in

neuroblastomas, the DRT gene may play a role in neuroblastoma and small cell lung carcinoma (SCLC) tumorigenesis.

By fluorescence in situ hybridization, Saito *et al.* (Genomics 26: 382-384, 1995

- 5 PubMed ID : 7601466) demonstrated that the ERK gene is located in chromosomal region 1p36.1. They showed that the homologous genes are located on mouse 4D2.2-D3 and rat 5q36.13, both of which are regions with conserved linkage homology to human chromosome 1p.
- 10 The invention relates to the use of CASB616 polypeptides and polynucleotides as described in greater detail below. The invention relates especially to the use of CASB616 having the nucleotide and amino acid sequences set out in SEQ ID NO:1 or 3 and SEQ ID NO:2 or 4 respectively.
- 15 The invention further relates to uses of polynucleotides and polypeptides which have at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, most preferably at least 97-99% or exact identity to the sequences identified in SEQ ID NO:1 or 3 and SEQ ID NO:2 or 4.
- 20 CASB616 polypeptides and polynucleotides are believed to be important immunogens for specific prophylactic or therapeutic immunization against tumours, because they are specifically expressed or highly over-expressed in tumours compared to normal cells and can thus be targeted by antigen-specific immune mechanisms leading to the destruction of the tumour cell. They can also be used to diagnose the occurrence of tumour cells.
- 25 Furthermore, their inappropriate expression in certain circumstances can cause an induction of autoimmune, inappropriate immune responses, which could be corrected through appropriate vaccination using the same polypeptides or polynucleotides. In this respect the most important biological activities to our purpose are the antigenic and immunogenic activities of the polypeptide of the present invention. A polypeptide of the present
- 30 invention may also exhibit at least one other biological activity of a CASB616 polypeptide, which could qualify it as a target for therapeutic or prophylactic intervention different from that linked to the immune response.

In a first aspect of the invention there are provided uses for CASB616 polypeptides as well as
5 biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

The present invention further provides uses for:

- 10 (a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2 or 4;
- (b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to
15 SEQ ID NO:1 or 3 over the entire length of SEQ ID NO:1 or 3 respectively; or
- (c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2 or 4.

20

The invention also provides uses for immunogenic fragments of a CASB616 polypeptide, that is, a contiguous portion of the CASB616 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 4. That is to say, the fragment (if necessary when coupled to a carrier) is capable of
25 raising an immune response which recognises the CASB616 polypeptide. Such an immunogenic fragment may include, for example, the CASB616 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of CASB616 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85%
30 identity, preferably at least 90% identity, more preferably at least 95% identity, most preferably at least 97-99% identity, more preferably at least more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2 or 4 over the entire length of SEQ ID NO:2 or 4 respectively.

A fragment is a polypeptide having an amino acid sequence that is entirely the same as part but not all of any amino acid sequence of any polypeptide of the invention. As with CASB616 polypeptides, fragments may be "free-standing", or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region in a single larger polypeptide.

Preferred fragments include, for example, truncation polypeptides having a portion of an amino acid sequence of SEQ ID NO:2 or 4 or of a variant thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence. Degradation forms of the polypeptides of the invention produced by or in a host cell, are also preferred. Further preferred are fragments characterized by structural or functional attributes such as fragments that comprise beta-barrels, alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Further preferred fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO:2 or 4, or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO:2 or 4.

Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

The polypeptides, or immunogenic fragments, for use in the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production. Furthermore, addition of exogenous polypeptide or lipid

tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

In one aspect, the invention relates to the use of genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa.

Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

The proteins may be chemically conjugated, or expressed as recombinant fusion proteins allowing increased levels to be produced in an expression system as compared to non-fused protein. The fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion partner and expression enhancing partner.

Fusion partners include protein D from *Haemophilus influenzae* and the non-structural protein from influenza virus, NS1 (hemagglutinin). Another fusion partner is the protein known as LytA. Preferably the C terminal portion of the molecule is used. LytA is derived from *Streptococcus pneumoniae* which synthesize an N-acetyl-L-alanine amidase LytA, (coded by the lytA gene {Gene, 43 (1986) page 265-272}) an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LytA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E.coli* C- LytA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C- LytA fragment at its amino terminus has been described {Biotechnology: 10, (1992) page 795-798}. It is possible to use the repeat

portion of the LytA molecule found in the C terminal end starting at residue 178, for example residues 188 - 305.

5 The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.

10 Polypeptides for use in the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood
15 in the art.

In a further aspect, the present invention relates to uses for polynucleotides that encode CASB616 polypeptides, particularly polynucleotides that encode the polypeptide herein designated CASB616, for use in or in preparation of the vaccine compositions described
20 herein.

In a particularly preferred embodiment the polynucleotide comprises a region encoding CASB616 polypeptides comprising a sequence set out in SEQ ID NO:1 or 3 which includes a full length gene, or a variant thereof.

25 Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:1 or 3 a polynucleotide of the invention encoding CASB616 polypeptide may be obtained using standard cloning and screening methods, from a cDNA library derived from mRNA in cells of human colon cancer, lung cancer, uterine cancer and fetal tissues. Suitable techniques
30 are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). Polynucleotides as described in the invention can also be

obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

Moreover, the DNA sequence set out in SEQ ID NO:1 or 3 contains an open reading frame
5 encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:2
or 4 with a deduced molecular weight that can be calculated using amino acid residue
molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:1, between the start codon at nucleotide number 105 and
10 the stop codon which begins at nucleotide number 3066 of SEQ ID NO:1, encodes the
polypeptide of SEQ ID NO:2.

The polynucleotide of SEQ ID NO:3, between the start codon at nucleotide number 26 and the
stop codon which begins at nucleotide number 3191 of SEQ ID NO:3, encodes the
15 polypeptide of SEQ ID NO:4.

In a further aspect, the present invention provides uses for an isolated polynucleotide
comprising or consisting of:

- 20 (a) a polynucleotide sequence which has at least 85% identity, more preferably at least
90% identity, yet more preferably at least 95% identity, even more preferably at least 97-
99% or exact identity to SEQ ID NO:1 or 3 over the entire length of SEQ ID NO:1 or 3;
or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity,
more preferably at least 90% identity, yet more preferably at least 95% identity, even
25 more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID
NO:2 or 4 over the entire length of SEQ ID NO:2 or 4.

A polynucleotide encoding a polypeptide for use in the present invention, may be obtained by
a process which comprises the steps of screening an appropriate library under stringent
30 hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an
SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or
comprising the sequence of SEQ ID NO:1 or 3 or a fragment thereof; and isolating a full-
length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides uses for a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:1 or 3. Also provided by the invention are uses for a coding sequence for a mature polypeptide or a fragment thereof, by itself as well
5 as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence, or other fusion peptide portions. The polynucleotide may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences,
10 termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the
15 invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides for use with the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally
20 associated sequences that control gene expression.

The nucleotide sequence encoding CASB616 polypeptide of SEQ ID NO:2 or 4 may be identical to the polypeptide encoding sequence contained in nucleotides 105 to 3068 of SEQ ID NO:1, or the polypeptide encoding sequence contained in nucleotides 26 to 3193 of
25 SEQ ID NO:3, respectively. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2 or 4.

The term "polynucleotide encoding a polypeptide" as used herein encompasses
30 polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a polypeptide having an amino acid sequence set out in SEQ ID NO:2 or 4. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an

integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

- 5 The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:2 or 4. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.
- 10 Further particularly preferred embodiments are polynucleotides encoding CASB616 variants, that have the amino acid sequence of CASB616 polypeptide of SEQ ID NO:2 or 4 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of
- 15 CASB616 polypeptide.

- Further preferred for use in the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding CASB616 polypeptide having an amino acid sequence set out in SEQ ID NO:2 or 4, and polynucleotides that are complementary to
- 20 such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 90% identical over its entire length to a polynucleotide encoding CASB616 polypeptide and polynucleotides complementary thereto. In this regard, polynucleotides at least 95% identical over their entire length to the same are particularly preferred. Furthermore, those with at least 97% are highly preferred among those with at least
- 25 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

- Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID
- 30 NO:1 or 3.

In accordance with certain preferred embodiments of this invention there are provided uses of polynucleotides that hybridize, particularly under stringent conditions, to CASB616 polynucleotide sequences, such as the polynucleotides in SEQ ID NO:1 or 3.

5 The invention further relates to uses of polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to uses of polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least
10 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about
15 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

20 A coding region of a CASB616 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:1 or 3 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

25 There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman, *et al.*, *PNAS USA* 85: 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™
30 technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the

DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly to the existing DNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

The invention also provides uses for polynucleotides that encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

Recombinant polypeptides of the present invention may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to an expression system which comprises a polynucleotide of the present invention, to host cells which are genetically engineered with such expression systems and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention.

Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., Basic Methods in Molecular Biology (1986) and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). Preferred such methods include, for instance, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Preferably the proteins of the invention are coexpressed with thioredoxin in trans (TIT). Coexpression of thioredoxin in trans versus in cis is preferred to keep antigen free of thioredoxin without the need for protease. Thioredoxin coexpression eases the solubilisation of the proteins of the invention. Thioredoxin coexpression has also a significant impact on protein purification yield, on purified-protein solubility and quality.

Representative examples of appropriate hosts include bacterial cells, such as *Streptococci*, *Staphylococci*, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used, for instance, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector which is able to maintain, propagate or express a polynucleotide to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of

well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual (*supra*). Appropriate secretion signals may be incorporated into the desired polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals.

The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses. Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia, fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelan Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), Listeria, Salmonella, Shigella, BCG. These viruses and bacteria can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

Another important aspect of the invention relates to a method for inducing, re-inforcing or modulating an immunological response in a mammal which comprises inoculating the mammal with a fragment or the entire polypeptide or polynucleotide of the invention, adequate to produce antibody and/or T cell immune response for prophylaxis or for therapeutic treatment of cancer and autoimmune disease and related conditions. Yet another aspect of the invention relates to a method of inducing, re-inforcing or

modulating immunological response in a mammal which comprises, delivering a polypeptide of the present invention *via* a vector or cell directing expression of the polynucleotide and coding for the polypeptide *in vivo* in order to induce such an immunological response to produce immune responses for prophylaxis or treatment of
5 said mammal from diseases.

A further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces, re-inforces or modulates an immunological response in that mammal to a polypeptide of the present
10 invention wherein the composition comprises a polypeptide or polynucleotide of the invention or an immunological fragment thereof as herein before defined. The vaccine formulation may further comprise a suitable carrier. Since a polypeptide may be broken down in the stomach, it is preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations
15 suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed
20 ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use.

A further aspect of the invention relates to the *in vitro* induction of immune responses to a fragment or the entire polypeptide or polynucleotide of the present invention or a
25 molecule comprising the polypeptide or polynucleotide of the present invention, using cells from the immune system of a mammal, and reinfusing these activated immune cells of the mammal for the treatment of disease. Activation of the cells from the immune system is achieved by *in vitro* incubation with the entire polypeptide or polynucleotide of the present invention or a molecule comprising the polypeptide or polynucleotide of the
30 present invention in the presence or absence of various immunomodulator molecules.

A further aspect of the invention relates to the immunization of a mammal by administration of antigen presenting cells modified by *in vitro* loading with part or the entire polypeptide of the present invention or a molecule comprising the polypeptide of

the present invention and administered *in vivo* in an immunogenic way. Alternatively, antigen presenting cells can be transfected *in vitro* with a vector containing a fragment or the entire polynucleotide of the present invention or a molecule comprising the polynucleotide of the present invention, such as to express the corresponding polypeptide, and administered *in vivo* in an immunogenic way.

The vaccine formulation of the invention may also include adjuvant systems for enhancing the immunogenicity of the formulation. Preferably the adjuvant system raises preferentially a TH1 type of response.

10

An immune response may be broadly distinguished into two extreme categories, being a humoral or cell mediated immune responses (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

15

Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

20

It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

25

The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and

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Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173*). Traditionally, TH1-type responses are associated with the production of the INF- γ and IL-2 cytokines by T-lymphocytes. Other cytokines often
5 directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2- type responses are associated with the secretion of IL-4, IL-5, IL-6 and IL-13.

It is known that certain vaccine adjuvants are particularly suited to the stimulation of
10 either TH1 or TH2 - type cytokine responses. Traditionally the best indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

15 Thus, a TH1-type adjuvant is one which preferentially stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and promotes development of both CD8+ cytotoxic T lymphocytes and antigen specific immunoglobulin responses associated with TH1-type isotype.

20 Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known
25 from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

30 Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (European Patent number 0 689 454).

3D-MPL will be present in the range of 10 μ g - 100 μ g preferably 25-50 μ g per dose wherein the antigen will typically be present in a range 2-50 μ g per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with an carrier.

5

The method of production of QS21 is disclosed in US patent No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown
10 to be successful TH1 stimulating adjuvants when formulated together with an antigen.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

15

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1;
20 preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5 : 1 to 1 : 1 3D-MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium
25 phosphate or aluminium hydroxide.

A preferred oil-in-water emulsion comprises a metabolisable oil, such as squalene, alpha tocopherol and Tween 80. In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an
30 emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1µg - 200µg, such as 10-100µg, preferably 10µg - 50µg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The present invention also provides a polyvalent vaccine composition comprising a vaccine formulation of the invention in combination with other antigens, in particular antigens useful for treating cancers, autoimmune diseases and related conditions. Such a polyvalent vaccine composition may include a TH-1 inducing adjuvant as hereinbefore described.

This invention also relates to the use of polynucleotides, in the form of primers derived from the polynucleotides of the present invention, and of polypeptides, in the form of antibodies or reagents specific for the polypeptide of the present invention, as diagnostic reagents.

The identification of genetic or biochemical markers in blood or tissues that will enable the detection of very early changes along the carcinogenesis pathway will help in determining the best treatment for the patient. Surrogate tumour markers, such as polynucleotide expression, can be used to diagnose different forms and states of cancer. The identification of expression levels of the polynucleotides of the invention will be useful in both the staging of the cancerous disorder and grading the nature of the cancerous tissue. The staging process monitors the advancement of the cancer and is determined on the presence or absence of malignant tissue in the areas biopsied. The polynucleotides of the invention can help to perfect the staging process by identifying markers for the aggressivity of a cancer, for

example the presence in different areas of the body. The grading of the cancer describes how closely a tumour resembles normal tissue of its same type and is assessed by its cell morphology and other markers of differentiation. The polynucleotides of the invention can be useful in determining the tumour grade as they can help in the determination of the differentiation status of the cells of a tumour. On the other hand, the polypeptide of the invention can be produced by stroma cells, in which cases, its specific expression or differential expression is a marker of disease conditions.

The diagnostic assays offer a process for diagnosing or determining a susceptibility to cancers, autoimmune disease and related conditions through diagnosis by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of polypeptide or mRNA. This method of diagnosis is known as differential expression. The expression of a particular gene is compared between a diseased tissue and a normal tissue. A difference between the polynucleotide-related gene, mRNA, or protein in the two tissues is compared, for example in molecular weight, amino acid or nucleotide sequence, or relative abundance, indicates a change in the gene, or a gene which regulates it, in the tissue of the human that was suspected of being diseased.

Decreased or increased expression can be measured at the RNA level. PolyA RNA is first isolated from the two tissues and the detection of mRNA encoded by a gene corresponding to a differentially expressed polynucleotide of the invention can be detected by, for example, in situ hybridization in tissue sections, reverse transcriptase-PCR, using Northern blots containing poly A+ mRNA, or any other direct or indirect RNA detection method. An increased or decreased expression of a given RNA in a diseased tissue compared to a normal tissue suggests that the transcript and/or the expressed protein has a role in the disease. Thus detection of a higher or lower level of mRNA corresponding to SEQ ID NO 1 or 3 relative to normal level is indicative of the presence of cancer in the patient.

mRNA expression levels in a sample can be determined by generation of a library of expressed sequence tags (ESTs) from the sample. The relative representation of ESTs in the library can be used to assess the relative representation of the gene transcript in the

starting sample. The EST analysis of the test can then be compared to the EST analysis of a reference sample to determine the relative expression levels of the polynucleotide of interest.

- 5 Other mRNA analyses can be carried out using serial analysis of gene expression (SAGE) methodology (Velculescu et. Al. Science (1995) 270:484) , differential display methodology (For example, US 5,776,683) or hybridization analysis which relies on the specificity of nucleotide interactions.
- 10 Alternatively, the comparison could be made at the protein level. The protein sizes in the two tissues may be compared using antibodies to detect polypeptides in Western blots of protein extracts from the two tissues. Expression levels and subcellular localization may also be detected immunologically using antibodies to the corresponding protein. Further assay techniques that can be used to determine levels of a protein, such as a polypeptide of
- 15 the present invention, in a sample derived from a host are well-known to those of skill in the art. A raised or decreased level of polypeptide expression in the diseased tissue compared with the same protein expression level in the normal tissue indicates that the expressed protein may be involved in the disease.
- 20 In the assays of the present invention, the diagnosis can be determined by detection of gene product expression levels encoded by at least one sequence set forth in SEQ ID NOS: 1 or 3. A comparison of the mRNA or protein levels in a diseased versus normal tissue may also be used to follow the progression or remission of a disease.
- 25 A large number of polynucleotide sequences in a sample can be assayed using polynucleotide arrays. These can be used to examine differential expression of genes and to determine gene function. For example, arrays of the polynucleotide sequences SEQ ID NO: 1 or 3 can be used to determine if any of the polynucleotides are differentially expressed between a normal and cancer cell. In one embodiment of the invention, an array of
- 30 oligonucleotides probes comprising the SEQ ID NO: 1 or 3 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene

expression, genetic linkage, and genetic variability (see for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

5 “Diagnosis” as used herein includes determination of a subject’s susceptibility to a disease, determination as to whether a subject presently has the disease, and also the prognosis of a subject affected by the disease.

The present invention, further relates to a diagnostic kit for performing a diagnostic assay which comprises:

- 10 (a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO: 1 or 3, or a fragment thereof ;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO: 2 or 4, or a fragment thereof; or
- 15 (d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2 or 4.

The nucleotide sequences of the present invention are also valuable for chromosomal localisation. The sequence is specifically targeted to, and can hybridize with, a particular
20 location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick,
25 Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined.

30

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them, can also be used as immunogens to produce antibodies immunospecific for polypeptides of the present invention. The term “immunospecific” means that the antibodies

have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

In a further aspect the invention provides an antibody immunospecific for a polypeptide
5 according to the invention or an immunological fragment thereof as hereinbefore defined.
Preferably the antibody is a monoclonal antibody

Antibodies generated against polypeptides of the present invention may be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal,
10 preferably a non-human animal, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., Nature (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, Immunology Today (1983) 4:72) and the EBV-hybridoma
15 technique (Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies, such as those described in U.S. Patent No. 4,946,778, can also be adapted to produce single chain antibodies to polypeptides
20 of this invention. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.
25 The antibody of the invention may also be employed to prevent or treat cancer, particularly ovarian and colon cancer, autoimmune disease and related conditions.

Another aspect of the invention relates to a method for inducing or modulating an immunological response in a mammal which comprises inoculating the mammal with a
30 polypeptide of the present invention, adequate to produce antibody and/or T cell immune response to protect or ameliorate the symptoms or progression of the disease. Yet another aspect of the invention relates to a method of inducing or modulating immunological response in a mammal which comprises, delivering a polypeptide of the

present invention *via* a vector directing expression of the polynucleotide and coding for the polypeptide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

- 5 It will be appreciated that the present invention therefore provides a method of treating abnormal conditions such as, for instance, cancer and autoimmune diseases, in particular, ovarian and colon cancer, related to either a presence of, an excess of, or an under-expression of, CASB616 polypeptide activity.
- 10 The present invention further provides for a method of screening compounds to identify those which stimulate or which inhibit the function of the CASB616 polypeptide. In general, agonists or antagonists may be employed for therapeutic and prophylactic purposes for such diseases as hereinbefore mentioned. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product
- 15 mixtures. Such agonists, antagonists or inhibitors so-identified may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide; or may be structural or functional mimetics thereof (see Coligan *et al.*, Current Protocols in Immunology 1(2):Chapter 5 (1991)). Screening methods will be known to those skilled in the art. Further screening methods may be found in for example D. Bennett *et al.*, J Mol
- 20 Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995) and references therein.

- Thus the invention provides a method for screening to identify compounds which stimulate or which inhibit the function of the polypeptide of the invention which comprises a method
- 25 selected from the group consisting of:
- (a) measuring the binding of a candidate compound to the polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;
 - (b) measuring the binding of a candidate compound to the polypeptide (or to the cells or
- 30 membranes bearing the polypeptide) or a fusion protein thereof in the presense of a labeled competitor;

- (c) testing whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells or cell membranes bearing the polypeptide;
- (d) mixing a candidate compound with a solution containing a polypeptide of claim 1, to
5 form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard; or
- (e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay.

10

The polypeptide of the invention may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. Well known screening methods may also be used to identify agonists and antagonists of the polypeptide of the invention which compete with the binding of the polypeptide of the
15 invention to its receptors, if any.

Thus, in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for polypeptides of the present invention; or compounds which decrease or enhance the production of such
20 polypeptides, which comprises:

- (a) a polypeptide of the present invention;
- (b) a recombinant cell expressing a polypeptide of the present invention;
- (c) a cell membrane expressing a polypeptide of the present invention; or
- (d) antibody to a polypeptide of the present invention;
- 25 which polypeptide is preferably that of SEQ ID NO:2.

It will be readily appreciated by the skilled artisan that a polypeptide of the present invention may also be used in a method for the structure-based design of an agonist, antagonist or inhibitor of the polypeptide, by:

30 (a) determining in the first instance the three-dimensional structure of the polypeptide;

(b) deducing the three-dimensional structure for the likely reactive or binding site(s) of an agonist, antagonist or inhibitor;

- (c) synthesising candidate compounds that are predicted to bind to or react with the deduced binding or reactive site; and
- (d) testing whether the candidate compounds are indeed agonists, antagonists or inhibitors.

5

Gene therapy may also be employed to effect the endogenous production of CASB616 polypeptide by the relevant cells in the subject. For an overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

10

Vaccine preparation is generally described in Pharmaceutical Biotechnology, Vol.61 Vaccine Design - the subunit and adjuvant approach, edited by Powell and Newman, Plenum Press, 1995. New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

15

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed. Generally, it is expected that each dose will comprise 1-1000 μ g of protein, preferably 2-100 μ g, most preferably 4-40 μ g. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive a boost in about 4 weeks.

20

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide

25

separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

5 "Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double stranded regions.

10 "Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of
15 a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue
20 may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

25 "Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known
30 methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds.,

Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

The preferred algorithm used is FASTA. The preferred parameters for polypeptide or polynucleotide sequence comparison using this algorithm include the following:

Gap Penalty: 12

Gap extension penalty: 4

Word size: 2, max 6

Preferred parameters for polypeptide sequence comparison with other methods include the following:

1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA.* 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

5

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polynucleotide comparisons.

- 10 By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and
- 15 wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the
- 20 numerical percent of the respective percent identity (divided by 100) and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y),$$

- wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, and y is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90
- 25 for 90%, 0.95 for 95%, etc., and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

30

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is be 100% identical, or it may include up to a

certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

"Homolog" is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a subject sequence. Such relatedness may be quantified by determining the degree of identity and/or similarity between the sequences being compared as hereinbefore described. Falling within this generic term are the terms "ortholog", meaning a polynucleotide or polypeptide that is the functional equivalent of a polynucleotide or polypeptide in another species and "paralog" meaning a functionally similar sequence when considered within the same species.

EXAMPLES

Example 1 Real-time RT-PCR analysis

Real-time RT-PCR (U. Gibson. 1996. Genome Research: 6,996) is used to compare
5 mRNA transcript abundance of the candidate antigen in matched tumour and normal
colon tissues from multiple patients. In addition, mRNA levels of the candidate gene in a
panel of normal tissues are evaluated by this approach.

Total RNA from normal and tumour colon is extracted from snap frozen biopsies using
TriPure reagent (Boehringer). Total RNA from normal tissues is purchased from
10 InVitrogen or is extracted from snap frozen biopsies using TriPure reagent. Poly-A⁺
mRNA is purified from total RNA after DNAase treatment using oligo-dT magnetic
beads (Dynal). Quantification of the mRNA is performed by spectrofluorimetry
(VersaFluor, BioRad) using SybrII dye (Molecular Probes). Primers for real-time PCR
amplification are designed with the Perkin-Elmer Primer Express software using default
15 options for TaqMan amplification conditions.

Real-time reactions are assembled according to standard PCR protocols using 2 ng of
purified mRNA for each reaction. SybrI dye (Molecular Probes) is added at a final
dilution of 1/75000 for real-time detection. Amplification (40 cycles) and real-time
detection is performed in a Perkin-Elmer Biosystems PE7700 system using conventional
20 instrument settings. Ct values are calculated using the PE7700 Sequence Detector
software. Two Ct values are obtained for each patient sample: the tumour Ct (CtT) and
the matched normal colon Ct (CtN). Ct values obtained by real-time PCR are log-linearly
related to the copy number of the target template. As the efficiency of PCR amplification
under the prevailing experimental conditions is close to the theoretical amplification
25 efficiency, $2^{(CtN-CtT)}$ is an estimate of the relative transcript levels in the two tissues (i.e.
fold mRNA over-expression in tumor). Real-time PCR reactions are performed on
biopsies from 17 patients. The level of mRNA over-expression is calculated as described
for each patient. The average level of mRNA over-expression for the candidate antigen
and the proportion of patients over-expressing the candidate antigen is then calculated
30 from this data set.

For normal tissues, Ct values for the candidate antigen are compared to those of actin
obtained with the same tissue sample.

Real-time PCR results in colon cancer/normal colon sample**Summary**

Patients over-expressing CASB616 in colon tumours (%)	Average level of over-expression in colon tumours (fold)
17/17 (100%)	17

5 Details of the 3 experiments

Experiment 1 (6 samples)						
	CtN (range)	CtN (mean)	CtT (range)	CtT (mean)	(CtN-CtT) (mean)	T over- expression (mean fold)
CASB616*						
Probe 1	30-34.5	32.3	28.5-31	29.8	2.5	5.7
Probe 2		26.3		26	4	16
Probe 3		26.6		27.1	2.7	6.5
Experiment 2 (5 samples)						
	CtN (range)	CtN (mean)	CtT (range)	CtT (mean)	(CtN-CtT) (mean)	T over- expression (mean fold)
CASB616	34-39	37.5	28-34.5	31	4.7	26
Experiment 3 (6 samples)						
	CtN (range)	CtN (mean)	CtT (range)	CtT (mean)	(CtN-CtT) (mean)	T over- expression (mean fold)
CASB616	38-40	39	29.5-35.5	32.5	7.7	207

* in the first experiment, 3 different probe pairs were used

Real-time PCR results in normal tissues**Ct values**

	<i>Bl</i>	<i>Bra</i>	<i>Sk</i>	<i>Ce</i>	<i>He</i>	<i>Ki</i>	<i>Li</i>	<i>Lu</i>	<i>Sp</i>	<i>Pl</i>	<i>Re</i>	<i>Te</i>
CASB616	31.5	29.5	20	32.5	34.5	31.5	30.5	31	30	29.5	28.5	31
Actin	16	17	18.5	17.5	17.5	17	15.5	16.5	30	16	16.5	16.5

	<i>Ao</i>	<i>Ad</i>	<i>Lu 2</i>	<i>Pr</i>	<i>Te 2</i>	<i>Oe</i>	<i>St</i>	<i>Co</i>	<i>Sk</i>	<i>Ov</i>	<i>Fa</i>	<i>Ce 2</i>
	<i>Gl</i>								<i>Mu</i>		<i>Tu</i>	
CASB616	24	30	21	29	22.5	33.5	30.5	22	30.5	24.5	25.5	28.5

Actin	40	28.5	28.5	27	25.5	26	28	33.5	29	30.5	28.5	23.5
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Bl: bladder; Bra: brain; Sk: skin; Ce: cervix; He: heart; Ki: kidney; Li: liver; Lu: lung; Sp: spleen; Pl: placenta; Re: rectum; Te: testis; Ao: aorta; Ad Gl: adrenal gland; Pr: prostate; Oe: esophagus; St: stomach; Sk Mu: skeletal muscle; Ov: ovary; Fa Tu: fallopian tube. Different samples of the same tissue have an additional numerical identifier.

Conclusion. CASB616 gene transcript is significantly over-expressed relative to normal colon in all primary colorectal tumors examined. Expression in the majority of normal tissues appears to be very low.

Example 2.

DNA microarrays

DNA micro-arrays are used to examine mRNA expression profiles of large collections of genes in multiple samples. This information is used to complement the data obtained by real-time PCR and provides an independent measure of gene expression levels in tumors and normal tissues.

Examples of current technologies for production of DNA micro-arrays include 1) The Affymetrix "GeneChip" arrays in which oligonucleotides are synthesized on the surface of the chip by solid phase chemical synthesis using a photolithographic process 2) DNA spotting technology in which small volumes of a DNA solution are robotically deposited and then immobilized onto the surface of a solid phase (e.g. glass). In both instances, the chips are hybridized with cDNA or cRNA which has been extracted from the tissue of interest (e.g. normal tissue, tumour etc...) and labeled with radioactivity or with a fluorescent reporter molecule. The labeled material is hybridized to the chip and the amount of probe bound to each sequence on the chip is determined using a specialized scanner. The experiment can be set-up with a single fluorescent reporter (or radioactivity) or, alternatively, can be performed using two fluorescent reporters. In this latter case, each of the two samples is labeled with one of the reporter molecules. The two labeled samples are then hybridized competitively to the sequences on the DNA chip. The ratio of the two fluorescent signals is determined for each sequence on the chip. This ratio is used to calculate the relative abundance of the transcript in the two samples. Detailed protocols are available from a number of sources including "DNA Microarrays: A

practical approach. Schena M. Oxford University Press 1999" and the World Wide Web (<http://cmgm.stanford.edu/pbrown/protocols/index.html>), <http://arrayit.com/DNA-Microarray-Protocols/>) and specialized distributors (e.g. Affymetrix).

Example 3.

5 EST profiles

A complementary approach to experimental antigen tissue expression characterization is to explore the human "Expressed Sequence Tags" (ESTs) database. ESTs are small fragments of cDNA made from a collection of mRNA extracted from a particular tissue or cell line. Such database currently provide a massive amount of ESTs (10^6) from
 10 several hundreds of cDNA tissue libraries, including tumoral tissues from various types and states of disease. By means of informatics tools, a comparison search of the CASB616 sequence is performed in order to have further insight into tissue expression.

Results of the database search using CASB616 sequence

DBEST	ATG Lib ID	Description	Category*
NCBI:580826	424	fetal heart	F
NCBI:581388	424	fetal heart	F
NCBI:584556	424	fetal heart	F
NCBI:1160708	882	NCI_CGAP_Co3 (12 pooled tumor colon)	TC
NCBI:1150180	895	NCI_CGAP_Br2 (pooled breast tumor tissues)	TB
NCBI:1150189	895	NCI_CGAP_Br2 (pooled breast tumor tissues)	TB
NCBI:1150229	895	NCI_CGAP_Br2 (pooled breast tumor tissues)	TB
NCBI:1150370	895	NCI_CGAP_Br2 (pooled breast tumor tissues)	TB
NCBI:1777532	843	Fetus	F
NCBI:2127996	1541	NCI_CGAP_Lu25	Tlg
NCBI:2129674	1541	NCI_CGAP_Lu25	Tlg
NCBI:2129684	1541	NCI_CGAP_Lu25	Tlg
NCBI:2649403	1540	NCI_CGAP_Co16	TC
NCBI:3415191	1918	Schneider fetal brain 00004	F
NCBI:24019	2	fetal brain	F
NCBI:1942	2	fetal brain	F
NCBI:79438	226	Human Placenta	N
NCBI:982827	695	fetal kidney	F

15 * F: fetal tissue libraries; TC: colon tumor libraries; TB: breast tumor libraries; Tlg: lung tumor libraries; N: normal tissue libraries.

Example 4

Northern-Southern blot analysis

Limited amounts of mixed tumour and matched normal colon cDNA are amplified by Advantage PCR (see above). Messenger RNA from multiple normal tissues is also amplified using the same procedure. The amplified cDNA (1 µg) is electrophoresed on a 1.2% agarose gel and transferred onto a nylon membrane. The membrane is hybridised
5 (AlkPhos Direct System) with a probe prepared using a fragment of the candidate TAA cDNA. Northern-Southern analysis provides information on transcript size, presence of splice variants and transcript abundance in tumour and normal tissues.

Example 5

10 Northern Blot Analysis

Northern blots are produced according to standard protocols using 1 µg of poly A+ mRNA. Radioactive probes are prepared using the Ready-to-Go system (Pharmacia).

Example 6 :

15

6.1 Expression and purification of tumour-specific antigens

Expression in microbial hosts is used to produce the antigen of the invention for vaccine purposes and to produce protein fragments or whole protein for rapid purification and generation of antibodies needed for characterization of the naturally expressed protein by
20 immunohistochemistry or for follow-up of purification.

Recombinant proteins may be expressed in two microbial hosts, *E. coli* and in yeast (such as *Saccharomyces cerevisiae* or *Pichia pastoris*). *Pichia*. This allows the selection of the expression system with the best features for this particular antigen production. In general, the recombinant antigen will be expressed in *E. coli* and the reagent protein
25 expressed in yeast.

The expression strategy first involves the design of the primary structure of the recombinant antigen. In general an expression fusion partner (EFP) is placed at the N terminal extremity to improve levels of expression that could also include a region useful for modulating the immunogenic properties of the antigen, an immune fusion partner
30 (IFP). In addition, an affinity fusion partner (AFP) useful for facilitating further purification is included at the C-terminal end.

When the recombinant strains are available, the recombinant product is characterized by the evaluation of the level of expression and the prediction of further solubility of the protein by analysis of the behavior in the crude extract.

After growth on appropriate culture medium and induction of the recombinant protein expression, total extracts are analyzed by SDS-PAGE. The recombinant proteins are visualized in stained gels and identified by Western blot analysis using specific antibodies.

A comparative evaluation of the different versions of the expressed antigen will allow the selection of the most promising candidate that is to be used for further purification and immunological evaluation.

The purification work follows a classical approach based on the presence of an His affinity tail in the recombinant protein. In a typical experiment the disrupted cells are filtered and the acellular extracts loaded onto an Ion Metal Affinity Chromatography (IMAC; Ni⁺⁺NTA from Qiagen) that will specifically retain the recombinant protein.

The retained proteins are eluted by 0-500 mM Imidazole gradient (possibly in presence of a detergent) in a phosphate buffer. This step is optimally followed by an Anion Exchange resin step and a Size Exclusion chromatography step depending on the success of the Imac step and the nature of the contaminants.

6.2 Antibody production and immunohistochemistry

Small amounts of relatively purified protein can be used to generate immunological tools in order to

- a) detect the expression by immunohistochemistry in normal or cancer tissue sections;
- b) detect the expression, and to follow the protein during the purification process (ELISA/ Western Blot); or
- c) characterise/ quantify the purified protein (ELISA).

6.2.1 Polyclonal antibodies:

Immunization

- 2- 3 Rabbits are immunized , intramuscularly (I.M.) , 3 times at 3 weeks intervals with 100µg of protein, formulated in the adjuvant 3D-MPL/QS21 . 3 weeks after each immunisation a blood sample is taken and the antibody titer estimated in the serum by ELISA using the protein as coating antigen following a standard protocol.

ELISA

96 well microplates (maxisorb Nunc) are coated with 5µg of protein overnight at 4°C. After 1 hour saturation at 37°C with PBS NCS 1%, serial dilution of the rabbit sera is added for 1H 30 at 37°C (starting at 1/10). After 3 washings in PBS Tween, anti rabbit biotinylated anti serum (Amersham) is added (1/5000). Plates are washed and peroxidase coupled streptavidin (1/5000) is added for 30 min at 37°C. After washing, 50µl TMB (BioRad) is added for 7 min and the reaction then stopped with H₂SO₄ 0.2M. The OD can be measured at 450 nm and midpoint dilutions calculated by SoftmaxPro.

10

6.2.2 Monoclonal antibodies:

Immunization

5 BALB/c mice are immunized 3 times at 3 week intervals with 5 µg of purified protein. Bleedings are performed 14 days post II and 1 week post 3. The sera is tested by Elisa on purified protein used as coated antigen. Based on these results (midpoint dilution > 10000) one mouse is selected for fusion

15

Fusion/ HATselection

Spleen cells are fused with the SP2/0 myeloma according to a standard protocol using PEG 40% and DMSO 5%. Cells are then seeded in 96 well plates $2.5 \times 10^4 - 10^5$ cells/well and resistant clones will be selected in HAT medium. The supernatant of these hybridomas will be tested for their content in specific antibodies and when positive, will be submitted to 2 cycles of limited dilution . After 2 rounds of screening, 3 hybridomas will be chosen for ascitis production.

20

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6.2.3 Immunohistochemistry

When antibodies are available, immuno staining is performed on normal or cancer tissue sections, in order to determine :

- the level of expression of the antigen of the invention in cancer relative to normal tissue or
- the proportion of cancer of a certain type expressing the antigen
- if other cancer types also express the antigen
- the proportion of cells expressing the antigen in a cancer tissue

30

Tissue sample preparation

After dissection, the tissue sample is mounted on a cork disk in OCT compound and rapidly frozen in isopentane previously super cooled in liquid nitrogen (-160°C). The block will then be conserved at -70°C until use. 7-10µm sections will be realized in a cryostat chamber (-20, -30°C).

Staining

Tissue sections are dried for 5 min at room Temperature (RT), fixed in acetone for 10min at RT, dried again, and saturated with PBS 0.5% BSA 5% serum. After 30 min at RT either a direct or indirect staining is performed using antigen specific antibodies. A direct staining leads to a better specificity but a less intense staining whilst an indirect staining leads to a more intense but less specific staining.

6.4 Analysis of human cellular immune responses to the antigen of the invention

The immunological relevance of the antigen of the invention can be assessed by *in vitro* priming of human T cells. All T cell lymphocyte lines and dendritic cells are derived from PBMCs (peripheral blood mononuclear cells) of healthy donors (preferred HLA-A2 subtype). An HLA-A2.1/K^b transgenic mice is also used for screening of HLA-A2.1 peptides.

Newly discovered antigen-specific CD8+ T cell lines are raised and maintained by weekly *in vitro* stimulation. The lytic activity and the γ-IFN production of the CD8 lines in response to the antigen or antigen derived-peptides is tested using standard procedures.

Two strategies to raise the CD8+ T cell lines are used: a peptide-based approach and a whole gene-based approach. Both approaches require the full-length cDNA of the newly discovered antigen in the correct reading frame to be either cloned in an appropriate delivery system or to be used to predict the sequence of HLA binding peptides.

Peptide-based approach

The HLA-A2 binding peptide sequences are predicted by the Parker's algorithm. Peptides are then screened in the HLA-A2.1/K^b transgenic mice model (Vitiello et al.). The

sequence used to perform the prediction is EPHB2v, as it is identical to EPHB2 with an additional C-terminal sequence extension.

Predicted epitopes binding the HLA_A0201 allele :

SEQ ID NO	Start Position	Subsequence Residue Listing	Score*
5	948	MMMEDILRV	2979.496
6	11	LLLPLLA AV	1006.209
7	387	GLTEPRIYI	235.260
8	712	RQNDGQFTV	167.692
9	22	TLMDSTTAT	113.047
10	641	KLPGkREIFV	1338.876
11	10	LLLLpLLAAV	1006.209
12	947	QMMMeDILRV	427.474
13	810	WSYGiVMWEV	115.285
14	554	FLIAvVVIAI	110.379

5 * Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence

Briefly, transgenic mice are immunized with adjuvanted HLA-A2 peptides, those unable to induce a CD8 response (as defined by an efficient lysis of peptide-pulsed autologous spleen cells) will be further analyzed in the human system.

10 Human dendritic cells (cultured according to Romani et al.) will be pulsed with peptides and used to stimulate CD8-sorted T cells (by FACS). After several weekly stimulations, the CD8 lines will be first tested on peptide-pulsed autologous BLCL (EBV-B transformed cell lines). To verify the proper *in vivo* processing of the peptide, the CD8 lines will be tested on cDNA-transfected tumour cells (HLA-A2 transfected LnCaP,
15 Skov3 or CAMA tumour cells).

Whole gene-based approach

CD8+ T cell lines will be primed and stimulated with either gene-gun transfected dendritic cells, retrovirally transduced B7.1-transfected fibroblasts, recombinant pox
20 virus (Kim et al.) or adenovirus (Butterfield et al.) infected dendritic cells. Virus infected

cells are very efficient to present antigenic peptides since the antigen is expressed at high level but can only be used once to avoid the over-growth of viral T cells lines.

After alternated stimulations, the CD8 lines are tested on cDNA-transfected tumour cells
5 as indicated above. Peptide specificity and identity is determined to confirm the immunological validation.

References

- Vitiello et al. (L. Sherman), J. Exp. Med., J. Exp. Med, 1991, 173:1007-1015.
10 Romani et al., J. Exp. Med., 1994, 180:83-93.
Kim et al., J. Immunother., 1997, 20:276-286.
Butterfield et al., J. Immunol., 1998, 161:5607-5613.

All publications, including but not limited to patents and patent applications, cited in this
15 specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

SEQUENCE INFORMATION

SEQ ID NO:1

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1 aacaaaagct ggagctccac cgcggtggcg gccgctctag cccctgggtc ggcccacctc
61 tgaaggttcc agaatcgata gtgaattcgt ggggaagcgc agccatgggt ctgcggaggc
121 tggggggcgc gctgctgctg ctgccgctgc tcgccgccgt ggaagaaacg ctaatggact
181 ccactacagc gactgctgag ctgggctgga tgggtgcatcc tccatcaggg tgggaagagg
241 tgagtggcta cgatgagaac atgaacacga tccgcacgta ccaggtgtgc aacgtgtttg
301 agtcaagcca gaacaactgg ctacggacca agtttatccg gcgccgtggc gccaccgca
361 tccacgtgga gatgaagttt tcgggtgcgtg actgcagcag catccccagc gtgcttggtc
421 cctgcaagga gaccttcaac ctctattact atgaggctga ctttgactcg gccaccaaga
481 ccttccccaa ctggatggag aatccatggg tgaaggtgga taccattgca gccgacgaga
541 gcttctccca ggtggacctg ggtggccgcg tcatgaaaat caacaccgag gtgcggaagt
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661 cctcatcgc cgctgcgtgtc ttctaccgca agtgcccccg catcatccag aatggcgcca
721 tcttccagga aacctgtcg ggggtcgaga gcacatcgct ggtggcgact cggggcagct
781 gcatcgccaa tgcggaagag gtggatgtac ccatcaagct ctactgtaac ggggacggcg
841 agtggctggg gcccatcggg cgctgcatgt gcaaagcagg cttcgaggcc gttgagaatg
901 gcaccgtctg ccgaggttgt ccatctggga ctttcaaggc caaccaaggg gatgaggcct
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1141 ctccccgcga ctccggaggc cgagaggacc tcgtctacaa catcatctgc aagactgtg
1201 gctcgggccc ggggtgcctgc acccgctgcg gggacaatgt acagtacgca ccacgccagc
1261 taggcctgac cgagccacgc atttacatca gtgacctgct ggcccacacc cagtacacct
1321 tcgagatcca ggctgtgaac ggcgttactg accagagccc cttctcgcct cagttcgcct
1381 ctgtgaacat caccaccaac caggcagctc catcggcagt gtccatcatg catcaggtga
1441 gccgcaccgt ggacagcatt accctgtcgt ggtcccagcc agaccagccc aatggcgctga
1501 tcctggacta tgagctgcag tactatgaga aggaagctcag tgagtacaac gccacgcca
1561 taaaaagccc caccaacacg gtcaccgtgc agggcctcaa agccggcgcc atctatgtct
1621 tccaggtgcg ggcacgcacc gtggcaggct acgggcgcta cagcggaag atgtacttcc
1681 agaccatgac agaagccgag taccagacaa gcacccagga gaagtggcca ctcatcatcg
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1801 acagaagacg ggggtttgag cgtgctgact cggagtacac ggacaagctg caacactaca
1861 ccagtggcca catgacccca ggcataaga tctacatcga tccttccacc tacgaggacc
1921 ccaacgaggc agtgcgggag tttgccagg aaattgacat ctctgtgtc aaaattgagc
1981 aggtgatcgg agcaggggag tttggcgagg tctgcagtgg ccacctgaag ctgccaggca
2041 agagagagat ctttgtggcc atcaagacgc tcaagtcggg ctacacggag aagcagcgcc
2101 gggacttctt gagcgaagcc tccatcatgg gccagttcga ccatcccaac gtcacccacc
2161 tggaggggtg cgtgaccaag agcacacctg tgatgatcat caccagattc atggagaatg
2221 gctccctggt ctcccttctc cggcaaaacg atgggcagtt cacagtcac cagctggtgg
2281 gcatgcttcg gggcatcgca gctggcatga agtacctggc agacatgaac tatgttcacc
2341 gtgacctggc tgcccgcaac atcctcgtca acagcaacct ggtctgcaag gtgtcggact
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Claims

1. A vaccine composition comprising an effective amount of a polypeptide which polypeptide comprises an amino acid sequence which has at least 85% identity to the amino acid sequence of SEQ ID NO: 2 or 4 or to an immunogenic fragment thereof,
5 together with a pharmaceutically acceptable carrier.
2. A vaccine composition according to claim 1 wherein the amino acid sequence has at least 95% identity to the amino acid sequence of SEQ ID NO: 2 or 4 or to an immunogenic fragment thereof.
10
3. A vaccine composition comprising an effective amount of a polynucleotide which polynucleotide comprises a nucleotide sequence which has at least 85% identity to the nucleotide sequence of SEQ ID NO: 1, 3 or to a fragment thereof which encodes an immunogenic polypeptide, together with a pharmaceutically acceptable carrier.
15
4. A vaccine composition comprising an effective amount of antigen presenting cells, modified by in vitro loading with a polypeptide of SEQ ID NO:2 or 4, or genetically modified in vitro to express a polypeptide of SEQ ID NO:2 or 4 and a pharmaceutically effective carrier.
20
5. A vaccine as claimed in any one of claims 1 to 4 which additionally comprises a TH-1 inducing adjuvant.
6. A vaccine as claimed in claim 5 in which the TH-1 inducing adjuvant is selected from
25 the group of adjuvants comprising: 3D-MPL, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide.
7. An antibody immunospecific for the polypeptide or immunological fragment as defined in any one of claims 1 to 6.
30
8. A method for screening to identify compounds which stimulate or which inhibit the function of the polypeptide of SEQ ID NO: 2 or 4 which comprises a method selected from the group consisting of:

- (a) measuring the binding of a candidate compound to the said polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;
- (b) measuring the binding of a candidate compound to the said polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof in the presense of a labeled competitor;
- (c) testing whether the candidate compound results in a signal generated by activation or inhibition of the said polypeptide, using detection systems appropriate to the cells or cell membranes bearing the polypeptide;
- (d) mixing a candidate compound with a solution containing a polypeptide of SEQ ID NO: 2 or 4, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard; or
- (e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay.

15

9. A method for the treatment of a subject by immunoprophylaxis or therapy comprising *in vitro* induction of immune responses to a molecule of any one of SEQ ID NOS: 1 - 4, using *in vitro* incubation of the polypeptide of SEQ ID NO: 2 or 4 or the polynucleotide of SEQ ID NO: 1 or 3 with cells from the immune system of a mammal, and reinfusing these activated immune cells to the mammal for the treatment of disease.

20

10. A method as claimed in claim 9 wherein the treatment is for ovarian or colon cancer.

11. An agonist or antagonist to the polypeptide of SEQ ID NO: 2 or 4.

25

12. A compound which is:

- (a) an agonist or antagonist to the polypeptide of SEQ ID NO: 2 or 4;
 - (b) isolated polynucleotide of SEQ ID NO: 1 or 3; or
 - (c) a nucleic acid molecule that modulates the expression of the nucleotide sequence encoding the polypeptide of any one of SEQ ID NO: 2 or 4;
- for use in therapy.

30

13. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of a polypeptide of SEQ ID NO: 2 or 4 in a subject comprising analyzing for the presence or amount of said polypeptide in a sample derived from said subject.

5

14. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of a polynucleotide of SEQ ID NO: 1 or 3 in a subject comprising analyzing for the presence or amount of said polynucleotide in a sample derived from said subject.

10

15. A process for diagnosing the presence of colon cancer or a susceptibility to colon cancer in a subject related to expression or activity of a polypeptide of SEQ ID NO: 2 or 4 in a subject comprising analyzing for the presence or amount of said polypeptide in a sample derived from said subject.

15

16. A process for diagnosing the presence of colon cancer or a susceptibility to colon cancer in a subject related to expression or activity of a polynucleotide of SEQ ID NO: 1 or 3 in a subject comprising analyzing for the presence or amount of said polynucleotide in a sample derived from said subject.

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<212> PRT

<213> homo sapiens

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Tyr Asp Glu Asn Met Asn Thr Ile Arg Thr Tyr Gln Val Cys Asn Val
50 55 60
Phe Glu Ser Ser Gln Asn Asn Trp Leu Arg Thr Lys Phe Ile Arg Arg
65 70 75 80
Arg Gly Ala His Arg Ile His Val Glu Met Lys Phe Ser Val Arg Asp
85 90 95
Cys Ser Ser Ile Pro Ser Val Pro Gly Ser Cys Lys Glu Thr Phe Asn
100 105 110
Leu Tyr Tyr Tyr Glu Ala Asp Phe Asp Ser Ala Thr Lys Thr Phe Pro
115 120 125
Asn Trp Met Glu Asn Pro Trp Val Lys Val Asp Thr Ile Ala Ala Asp
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Glu Ser Phe Ser Gln Val Asp Leu Gly Gly Arg Val Met Lys Ile Asn
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Thr Glu Val Arg Ser Phe Gly Pro Val Ser Arg Ser Gly Phe Tyr Leu
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Ala Phe Gln Asp Tyr Gly Gly Cys Met Ser Leu Ile Ala Val Arg Val
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Phe Tyr Arg Lys Cys Pro Arg Ile Ile Gln Asn Gly Ala Ile Phe Gln
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Glu Thr Leu Ser Gly Ala Glu Ser Thr Ser Leu Val Ala Ala Arg Gly
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Ser Cys Ile Ala Asn Ala Glu Glu Val Asp Val Pro Ile Lys Leu Tyr
225 230 235 240
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245 250 255
Lys Ala Gly Phe Glu Ala Val Glu Asn Gly Thr Val Cys Arg Gly Cys
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 Gln Ala Ala Pro Ser Ala Val Ser Ile Met His Gln Val Ser Arg Thr
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 Tyr Asn Ala Thr Ala Ile Lys Ser Pro Thr Asn Thr Val Thr Val Gln
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 Gly Leu Lys Ala Gly Ala Ile Tyr Val Phe Gln Val Arg Ala Arg Thr
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 Ser Gly Tyr Thr Glu Lys Gln Arg Arg Asp Phe Leu Ser Glu Ala Ser
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 755 760 765
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<211> 1055

<212> PRT

<213> homo sapiens

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50      55      60
Phe Glu Ser Ser Gln Asn Asn Trp Leu Arg Thr Lys Phe Ile Arg Arg
65      70      75      80
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85      90      95
Cys Ser Ser Ile Pro Ser Val Pro Gly Ser Cys Lys Glu Thr Phe Asn
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115     120     125
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130     135     140
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145     150     155     160
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165     170     175
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 245 250 255
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 260 265 270
 Pro Ser Gly Thr Phe Lys Ala Asn Gln Gly Asp Glu Ala Cys Thr His
 275 280 285
 Cys Pro Ile Asn Ser Arg Thr Thr Ser Glu Gly Ala Thr Asn Cys Val
 290 295 300
 Cys Arg Asn Gly Tyr Tyr Arg Ala Asp Leu Asp Pro Leu Asp Met Pro
 305 310 315 320
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 325 330 335
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 340 345 350
 Arg Glu Asp Leu Val Tyr Asn Ile Ile Cys Lys Ser Cys Gly Ser Gly
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 385 390 395 400
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 420 425 430
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 645 650 655
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 660 665 670
 Ile Met Gly Gln Phe Asp His Pro Asn Val Ile His Leu Glu Gly Val
 675 680 685
 Val Thr Lys Ser Thr Pro Val Met Ile Ile Thr Glu Phe Met Glu Asn
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 705 710 715 720

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      770      775      780
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      785      790      795      800
Arg Lys Phe Thr Ser Ala Ser Asp Val Trp Ser Tyr Gly Ile Val Met
      805      810      815
Trp Glu Val Met Ser Tyr Gly Glu Arg Pro Tyr Trp Asp Met Thr Asn
      820      825      830
Gln Asp Val Ile Asn Ala Ile Glu Gln Asp Tyr Arg Leu Pro Pro Pro
      835      840      845
Met Asp Cys Pro Ser Ala Leu His Gln Leu Met Leu Asp Cys Trp Gln
      850      855      860
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      885      890      895
Ser Ser Gly Ile Asn Leu Pro Leu Leu Asp Arg Thr Ile Pro Asp Tyr
      900      905      910
Thr Ser Phe Asn Thr Val Asp Glu Trp Leu Glu Ala Ile Lys Met Gly
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Gln Tyr Lys Glu Ser Phe Ala Asn Ala Gly Phe Thr Ser Phe Asp Val
      930      935      940
Val Ser Gln Met Met Met Glu Asp Ile Leu Arg Val Gly Val Thr Leu
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Pro Arg Ala Thr Gly Arg Thr Lys Arg Cys Gln Pro Arg Asp Val Thr
      995      1000      1005
Lys Lys Thr Cys Asn Ser Asn Asp Gly Lys Lys Lys Gly Met Gly Lys
      1010      1015      1020
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<400> 14

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